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# An inherited defect in the C3 convertase, C3b,Bb, associated with glomerulonephritis

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**An inherited defect in the C3 convertase, C3b,Bb, associated with glomerulonephritis.** The control of the amplification C3 convertase, C3b,Bb, of the serum complement system has been found to be defective in five members of a family spanning three generations. One of the five has membranoproliferative glomerulonephritis (MPGN) type III and another has mild idiopathic rapidly progressive glomerulonephritis. The defect is manifested by low serum concentrations of C3 and usually factor B with normal levels of the proteins which control the convertase, H and I. C3 nephritic factor (C3NeF) was not demonstrable. Enhanced C3 conversion was produced by the incubation of their serum at 37°C for 30 min. This conversion was further accelerated by incubation after increasing the serum magnesium concentration by increments ranging from 0.25 to 1.9 mM. Incremental additions of H to serum depleted of H indicated that the amplification convertase of affected family members required more H for its inhibition than did that of normal subjects. This requirement was reduced by the addition of purified normal C3 but not by the addition of purified C3 of the propositus. It is postulated that affected family members are heterozygous for a gene producing an abnormal C3 which, as a constituent of the amplification convertase, C3b,Bb, confers resistance to H. Investigation of this apparently nephritogenic defect may provide insight into the pathogenesis of these glomerulonephritides.

**Déficit héréditaire de la C3 convertase, C3b,Bb, associé à une glomérulonéphrite.** Le contrôle de la convertase d'amplification de C3, C3b,Bb, du complément sérique a été trouvé défectueux chez cinq membres d'une famille couvrant trois générations. Un des cinq avait une glomérulonéphrite membrano-prolifératives (MPGN) de type III et un autre avait une glomérulonéphrite idiopathique rapidement progressive. Le défaut se manifeste par des concentrations faibles de C3 et habituellement de facteur B sériques avec des niveaux normaux des protéines contrôlant la convertase H et I. Le facteur néphritique C3 (C3NeF) n'était pas démontrable. Une augmentation de la C3 conversion a été produite par l'incubation de leur sérum à 37°C pendant 30 min. Cette conversion était encore plus accélérée par incubation avec augmentation de la concentration de magnésium sérique dans des valeurs comprises entre 0,25 et 1,9 mM. Des augmentations progressives d'H au sérum déplété en H ont montré que la convertase d'amplification des membres de la famille touchée nécessitait plus d'H pour son inhibition que cela était nécessaire aux sujets normaux. Ce besoin était réduit en ajoutant du C3 normal purifié, mais non du C3 purifié des propositus. Il est postulé que les membres des familles affectées sont hétérozygotes pour un gène produisant un C3 anormal, lequel, étant un constituant de la convertase d'amplification C3b,Bb, confère une résistance à H. L'investigation de ce défaut en apparence néphritogène pourrait fournir des informations sur la pathogénie de ces glomérulonéphrites.

Immune complex disease associated with deficiencies of serum complement components is being reported with increasing frequency. The association of homozygous C2 [1, 2] and C4 [3, 4] deficiencies and, occasionally, of homozygous deficiencies of terminal complement components [5] with systemic lupus erythematous (SLE) is well known and complete or subtotal deficiency of C2 has been associated with membranoproliferative glomerulonephritis (MPGN) [6–10]. Recently, patients with partial deficiencies of complement components have been reported to have immune complex disease. Glass et al [11] found heterozygous C2 deficiency to be significantly more frequent (5.1%) among patients with SLE than in the general population (1.6%); low levels of C3 have been associated with chronic glomerulonephritis. The low C3 levels have been the result of heterozygous deficiency [12], a hypomorphic variant of C3 (C3f) [13] or secondary to partial H deficiency [14].

The present paper reports a family (the P family) with a hitherto undescribed inherited defect of the complement system in which two of the affected family members have relatively rare forms of chronic glomerulonephritis. One sibling has membranoproliferative glomerulonephritis (MPGN) type III [15] and the other, idiopathic rapidly progressive glomerulonephritis [16]. Both children, as well as three other family members, consistently have low serum concentrations of C3 and usually have low concentrations of factor B. Although their C3 concentrations are in a range expected for heterozygous deficiency of this protein, functional studies of their complement system give evidence of a defect in the regulation of the amplification C3 convertase, C3b,Bb by the control protein, H. The presence of rare forms of chronic glomerulonephritis in two of the five affected family members suggests that this defect is nephritogenic.

## Methods

*Complement protein measurements.* Studies were done on serum from blood allowed to clot at room temperature or plasma (citrate phosphate dextrose adenine), both stored at −70°C. Measurements of C1q, C2, C4, C3, factor B, H, I, and C4 binding protein (C4bp) were by radial immunodiffusion (RID) [17, 18] using goat antiserum produced in this laboratory. Ring diameters were measured with a 10× binocular micro-

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scope with a reticule calibrated in millimeters in one eyepiece. Measurements on serum specimens from 163 healthy hospital personnel provided normal values.

*Circulating immune complexes (CIC).* CIC were measured by a modification [19] of the solid phase C1q method of Hay, Nineham, and Roitt [20]. Normal values were obtained from serum specimens of 20 healthy hospital personnel.

*Component minus regulatory protein levels (C−R).* The balance between the component and the regulatory proteins of the C3b feedback mechanism was described previously [18]. This balance is expressed by C−R in which C is the sum of the concentrations of the component proteins of the C3b feedback mechanism, C3 and factor B, expressed as percent of the average adult concentration, and R is the sum of the concentrations of the regulatory proteins of that feedback, H and I, expressed as a percent of the average concentration. The calculation used the same average concentrations as used previously [18]. Markedly negative values for C−R are seen when complement reactive material is present in the circulation.

*Measurement of C3 conversion and factor B cleavage.* In functional studies of the complement system, C3 conversion was quantitated by the loss of the B antigen of C3. This antigen is present only on intact C3 and not on breakdown products [21]. B antigen was measured by RID with antiserum made monospecific for the B determinant by absorption with aged serum. C3 conversion was expressed either as a percent of the initial B antigen level or as the fall in B antigen concentration in units per milliliter (U/ml).

Factor B cleavage was measured semiquantitatively by RID using an antiserum containing antibody to Ba, the alpha migrating breakdown product of factor B, in a concentration relative to that of other determinants, which resulted in the formation of a double precipitin ring with serum containing cleaved factor B. For the measurement, the area of the precipitin ring produced by untreated serum, calculated from the ring diameter, was subtracted from the area of the larger ring produced by the serum after it had reacted with a complement activator.

*C3 nephritic factor (C3NeF).* To test for C3NeF activity in serum from affected members of the P family (P family serum), the concentration of the B antigenic determinant of C3 was measured before and after incubation for 20 min at 37°C of a mixture of equal volumes of serum from family members and normal subjects.

*Experiments with complement activators.* The response of P family serum to complement activators was tested by measurement of C3 conversion and factor B cleavage. Zymosan was suspended in 0.15 M NaCl, centrifuged and the pellet containing 0.1 mg suspended in 0.1 ml of serum. A precipitate of bovine serum albumin (BSA-) antiBSA was prepared at equivalence and 0.01 mg added to 0.1 ml of serum. To increase the serum $Mg^{++}$ level, small volumes of $MgCl_2$ solution, 0.005 to 0.04 M, were added to 0.1 or 0.2 ml of serum. In all experiments, experimental and control reaction mixtures were brought to the same volume by the addition of 0.15 M NaCl. Samples for the measurements were taken after the addition of the activator to the serum cooled in ice and again after the serum was warmed to 37°C. Incubation at 37°C with zymosan and immune precipitate was for 5 min and with $Mg^{++}$ for variable periods up to 30 min.

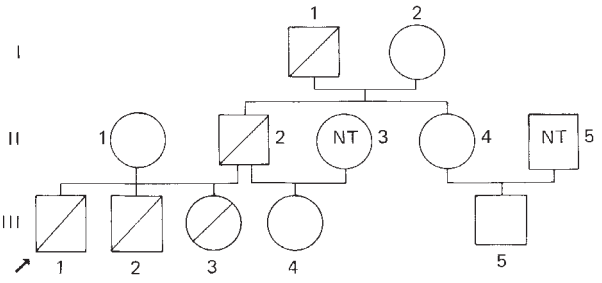


**Fig. 1.** *Pedigree of the P family.* The *diagonal lines* through the symbols indicate family members with low levels of C3 and factor B. The propositus is indicated by the *arrow*. His brother, III-2, also had glomerulonephritis. Abbreviation: NT, not tested.

*Complement component isolation.* H was isolated from normal human serum (NHS) containing 0.07 M EDTA and from EDTA P family serum by passage of the serum over a column of monospecific anti-H covalently coupled to Sepharose 4B [22]. The H was eluted with 3.5 M NaSCN and immediately dialyzed against phosphate-buffered saline. The isolated protein was not contaminated with other complement proteins as determined by RID using antiserum to C1q, C2 through C6, P, factor B and I.

I was isolated by an identical procedure which used monospecific anti-I coupled to Sepharose 4B. The purified preparation contained I in high concentration but did not contain any of the complement proteins listed above and it was devoid of H.

C3 was isolated from normal and P family plasma by the method of Hammer et al [23]. After gel filtration on Sepharose 6B, the material was passed over a Sepharose 4B column to which was linked antibody to IgG, IgA, IgM, C4, C5, H, and albumin. The final products showed precipitin lines representing only C3 when subjected to immunoelectrophoretic analysis with goat antiserum to whole human serum. No precipitin rings were seen when the preparations were tested by RID against antisera to H, I, P, C4bp, C1q, C4, C2, C5, C6, C7, C8, C9, IgG, IgA, IgM, and albumin. The C3 preparations were homogeneous by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

*Functional testing of H and I.* The EDTA NHS and EDTA P family serum from which H was removed by immunoabsorption, as described above, were found, after concentration to the original volume, to contain no H and were used as a reagent (RH) for functional testing of H. The EDTA NHS and EDTA P family serum used as a source of I was also found to be depleted of that protein and was used as a reagent (RI) for functional testing. By RID, the RH was found to contain I and the RI,H in normal concentrations. Both contained the other alternative pathway proteins in normal concentration but were devoid of Clq. Some preparations were dialyzed to remove EDTA before use. In all cases they were tested to determine the minimal amount of $Mg^{++}$, as $MgCl_2$, required to produce rapid C3 conversion and factor B cleavage. For functional tests of H or I, tubes containing aliquots of the appropriate reagent were cooled in an ice bath and increasing quantities of the missing protein were added, followed by the minimal amount of $Mg^{++}$ determined as above. While in the ice bath, samples were placed on RID plates for measurement of the B antigen of C3 and factor B cleavage. The tubes were then placed in a 37°C water bath and after 20 min the measurements were repeated.

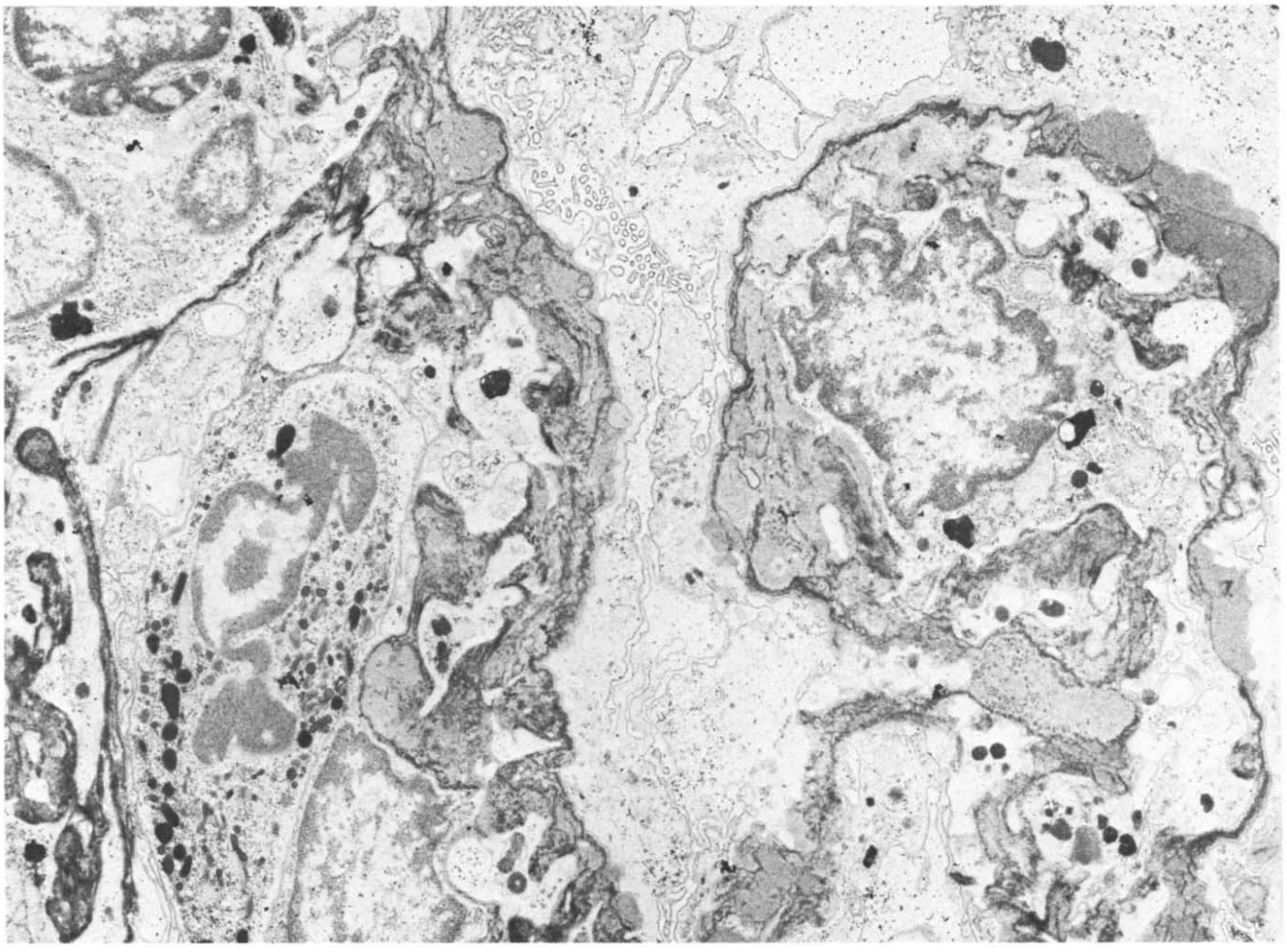

**Fig. 2.** *Silver impregnated electron micrograph of a glomerulus of the propositus (III-1).* Abundant deposit material is seen in the capillary loops occupying both subepithelial and subendothelial positions. There are numerous interruptions and duplications of lamina densa in relationship to the deposits, characteristic of MPGN type III. (×5900)

*Genetic polymorphism of C3.* To determine C3 electrophoretic variants, serum was subjected to high voltage electrophoresis by the method of Teisberg [24]. Serum samples containing known C3 variants, typed in the laboratory of Dr. Chester A. Alper (Center for Blood Research, Children's Hospital Medical Center, Boston, Massachusetts), were run concurrently with samples from patients.

*Statistical methods.* The significance of differences of means of small samples was calculated as described by Mainland [25].

### *Pedigree and clinical data*

The pedigree of the P family is shown in Figure 1. The propositus, III-1, has MPGN type III. His brother, III-2, has mild idiopathic rapidly progressive glomerulonephritis.

*III-1 (propositus).* This male Caucasian was referred in 1968, at age 12 years, with a history of hematuria and proteinuria of 2 years duration. Renal function was normal but the measurement of complement proteins indicated low concentrations of C3, C5, and P. Concentrations of C1q, C2, C4, factor B, H, I, and C4bp, measured on serum specimens stored at −70°C, were normal. By light microscopy, renal biopsy tissue showed changes characteristic of MPGN type III [15] and by electron microscopy, there were subendothelial and subepithelial deposits associated with a basement membrane which appeared disrupted and replicated (Fig. 2). By immunofluorescence, C3 and P were present in large amounts along glomerular capillary walls and IgG was found in deposits in proximity to the capillary waist. Treatment was with prednisone given on alternate days. A second renal biopsy 5 years later showed no evidence of histologic progression (patient 6, [26]). Ten years after diagnosis, the BUN was 22 mg/dl and the serum creatinine, 0.9 mg/dl. As shown in Table 1, in nine serum specimens obtained over a 14-year period, concentrations of C3 were consistently below the normal range. Concentrations of factor B, on the other hand, were within the lower limit of the normal range but the mean (15.4 mg/dl) was significantly lower than that for 163 normal subjects ($P < 0.001$). Values for C-R were markedly negative. In one specimen, Clq binding immune complexes were in a slightly elevated concentration. Concentrations of I, H, C4bp, and C2 were normal.

*III-2.* The brother was referred in 1978, at age 6 years, because of intermittent proteinuria in trace to 1+ amounts of several years duration. He also had recurrent upper respiratory infections. Serum C3 and factor B concentrations were low, but

**Table 1.** Levels of complement proteins and circulating immune complexes in the serum of P family members[a]

| Pedigree no. | Date | C3 | Factor B | I | H | C4bp %N | C2 mg/dl | C-R % | CIC μg/ml |
|---|---|---|---|---|---|---|---|---|---|
| | | mg/dl | | | | | | | |
| I-1 | 06-27-78 | *61* | *12.4* | 4.8 | 67.6 | — | 2.9 | *−142* | 1.8 |
| I-2 | 06-27-78 | 155 | 26.9 | 5.3 | 68.8 | — | 1.9 | − 23 | — |
| II-1 | 03-22-78 | 114 | 37 | 4.5 | 49.3 | — | 3.9 | + 56 | — |
| | 08-26-78 | 117 | 23 | 3.8 | 52.7 | 87 | 3.1 | + 2 | — |
| | 11-29-80 | 134 | 23 | 3.5 | 48 | 90 | 2.4 | + 26 | — |
| II-2 | 06-19-78 | *59* | *10.5* | 4.2 | 60.2 | — | *1.4* | *−125* | 19 |
| | 06-27-78 | *61* | *9.6* | 4.5 | 71.6 | — | *1.7* | *−155* | — |
| | 08-26-78 | *54* | *11.2* | 4.7 | 66.5 | 104 | *1.7* | *−149* | — |
| | 11-29-80 | *74* | 16 | 5.2 | 76 | 104 | *1.7* | *−142* | — |
| II-4 | 08-26-78 | 106 | 24.2 | 4.3 | 56.2 | — | 3.1 | − 26 | — |
| III-1 | 01-29-68 | *20* | 14.5 | 3.6 | 51.6 | — | 3.0 | *−104* | — |
| | 06-10-68 | *24* | 20 | 4.5 | 55 | 119 | 3.2 | *−105* | — |
| | 11-05-71 | *44* | 15.6 | 3.9 | 59.6 | — | 2.8 | *−103* | — |
| | 04-23-73 | *33* | 18 | 4.0 | 58.4 | — | 2.6 | *−101* | — |
| | 11-06-76 | *44* | 15.6 | 4.1 | — | — | — | — | — |
| | 05-14-77 | *51* | 14.6 | 3.8 | 61.9 | — | 2.7 | *−105* | — |
| | 08-26-78 | *46* | 14 | 3.5 | 55 | 98 | 2.6 | − *92* | 10.3 |
| | 06-09-80 | *47* | *12* | 3.3 | 53 | 95 | 2.4 | − *90* | — |
| | 05-10-82 | *58* | 14.4 | 3.5 | 48 | 103 | 2.8 | − *68* | — |
| III-2 | 01-09-78 | *64* | *9.6* | 3.4 | 56.1 | — | 2.4 | − *97* | — |
| | 03-20-78 | *52* | *11.4* | 3.7 | 54.4 | — | 2.9 | *−102* | — |
| | 08-26-78 | *44* | *10.6* | 3.5 | 52.7 | 87 | 2.6 | *−103* | 1.4 |
| | 12-22-78 | *24* | *12* | 3.7 | 51.0 | 100 | 3.8 | − *91* | — |
| | 11-29-80 | *44* | *7.3* | 2.8 | 46 | 82 | 2.1 | − *88* | — |
| | 06-30-81 | *56* | 16.4 | 4.5 | 57 | 115 | 2.7 | − *93* | — |
| III-3 | 06-19-78 | *76* | *8.8* | 4.3 | 60 | — | *1.1* | — | — |
| | 06-27-78 | *71* | *10.2* | 4.4 | 64.2 | — | *1.5* | *−129* | — |
| | 08-26-78 | *58* | *8.9* | 4.3 | 57.3 | 87 | *1.2* | *−129* | 8.0 |
| | 11-29-80 | *59* | *6.2* | 3.4 | 54 | 82 | *1.1* | *−112* | — |
| III-4 | 09-28-78 | 128 | 24 | 3.9 | 56.1 | 96 | 2.9 | + 1 | — |
| III-5 | 08-26-78 | 109 | 21 | 3.8 | 55 | — | 2.6 | − 7 | — |
| Normal range[b] | | 86 to 169 | 13.3 to 31.5 | 2.4 to 4.9 | 37 to 68 | 61 to 116 | 1.9 to 3.6 | ± 35 | < 8 |

Abbreviations: C-R, difference between sums of component and regulatory proteins of C3b amplification loop expressed as percent of normal (see **Methods** and [18]); CIC, circulating immune complexes.

[a] Abnormally low values are italicized.

[b] Normal range ($\bar{x} \pm 2$ SD) for complement components are based on measurements on serum from 163 healthy hospital personnel.

other complement proteins were in normal concentration. A renal biopsy specimen showed slight glomerular cellular proliferation with no adhesions or crescents. By immunofluorescence there were, however, numerous large mesangial deposits containing C3, C5, and P but no Ig (Fig. 3). Electron microscopy showed large subepithelial deposits located along the nonfiltering portion of the basement membrane at the capillary waist. The deposits were typical of those seen early in the course of idiopathic rapidly progressive glomerulonephritis [16]. His proteinuria disappeared without therapy, but 3 years later he had an episode of gross hematuria followed by persistent microhematuria and proteinuria. A second renal biopsy specimen revealed the same abnormalities as the first. Urinalysis was normal after 15 days of alternate day prednisone therapy. BUN and serum creatinine concentrations were always within the normal range. As shown in Table 1, complement measurements on 6 serum specimens obtained over a 3-year period have consistently shown low concentrations of C3 and, usually, factor B. The mean concentration of factor B was 11.1 mg/dl, significantly ($P < 0.001$) less than the mean of controls. With the exception of very negative C-R values, the concentrations of the other proteins were well within the normal range.

As indicated in Figure 1, the grandfather, father, and sister of the propositus also had persistently low serum concentrations of C3 and factor B and low values for C−R. The grandfather, I-1, had asthma and maturity onset diabetes but was not known to have hematuria, proteinuria, or other signs of renal disease. He died at age 70 of cardiac failure. The father and sister are in good health and urinalyses have shown no abnormality.

The results of measurements of complement proteins in serum specimens from these family members, as well as from unaffected members, are shown in Table 1. The table indicates

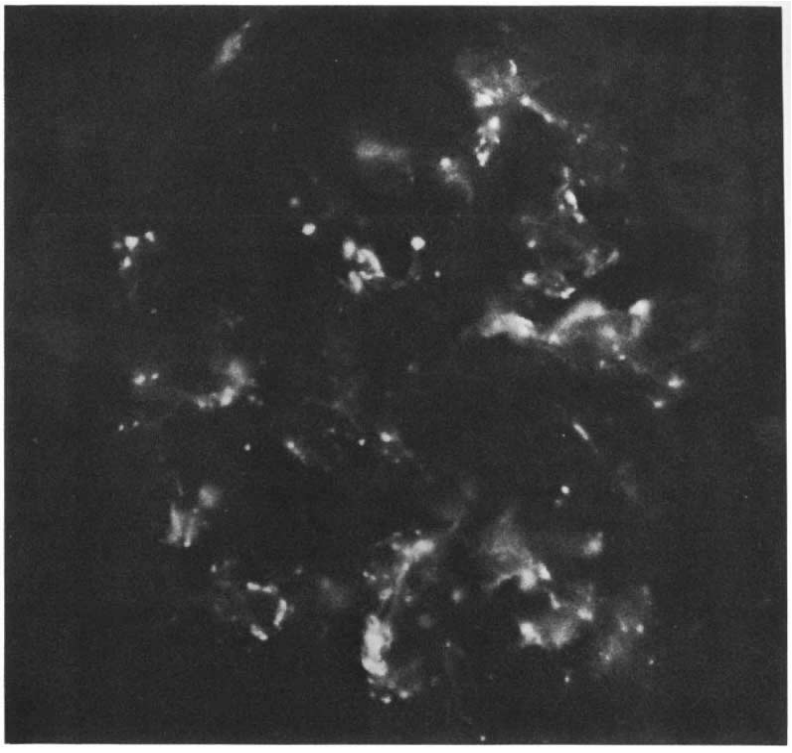

**Fig. 3.** *Immunofluorescence produced by labeled antiserum to C3 in the glomerulus of the brother of the propositus (III-2).* Numerous discrete deposits, some large, are present which, by electron microscopy, were subepithelial and at the capillary waist.

**Table 2.** Effect of complement activators in converting C3 in P family and normal serum. Incubation for 5 min at 37°C

| Serum source | No. subjects | Average initial B antigen μ/ml | Average B antigen loss | | Average B antigen loss | |
|---|---|---|---|---|---|---|
| | | | μ/ml | *P* | Percent of initial | *P* |
| BSA-anti BSA | | | | | | |
| P family[a] | 4 | 12.1 | 7.2 | | 59.3 | |
| NHS 1:1 | 9 | 16.9 | 6.9 | NS | 40.8 | < 0.01 |
| NHS neat | 9 | 36.8 | 12.0 | < 0.01 | 32.5 | < 0.01 |
| Zymosan | | | | | | |
| P family[a] | 4 | 12.7 | 6.7 | | 51.9 | |
| NHS 1:1 | 9 | 17.3 | 4.6 | NS | 28.9 | < 0.01 |
| NHS neat | 9 | 36.1 | 8.2 | NS | 22.8 | < 0.01 |

[a] Specimens from family members II-1, III-2, III-3, and the propositus, III-1.

that concentrations of C2 were low in the father and the sister and at the lower limit of normal in the grandmother. This deficiency appears unrelated to the depressed C3 and factor B concentrations.

## Results

Tests for complement reactive material which might account for the low C3 and factor B concentrations and the very negative C−R values in P family serum yielded negative or inconsistent results. C3NeF activity was not detectable. The levels of CIC were slightly above the normal range in the propositus and his father, who had no signs of renal disease, but in other affected family members were well within the normal range (Table 1). Levels of CIC measured by this method in other patients with MPGN have varied widely [19].

The effect of incubation of P family serum with various complement activators was extensively studied. In general, the response differed from that of normal serum in that it was accelerated. The results after a 5-min incubation of the serum with zymosan and BSA-anti BSA are shown in Table 2. Values are given for the loss of B antigen both in units per milliliter and as a percent of initial B antigen concentration. Expressed as units per milliliter, B antigen loss with either activator in P family serum was not significantly different from that of normal serum. In fact, B antigen loss in undiluted NHS incubated with BSA-anti BSA averaged significantly greater than in P family serum. On the other hand, when B antigen loss was expressed

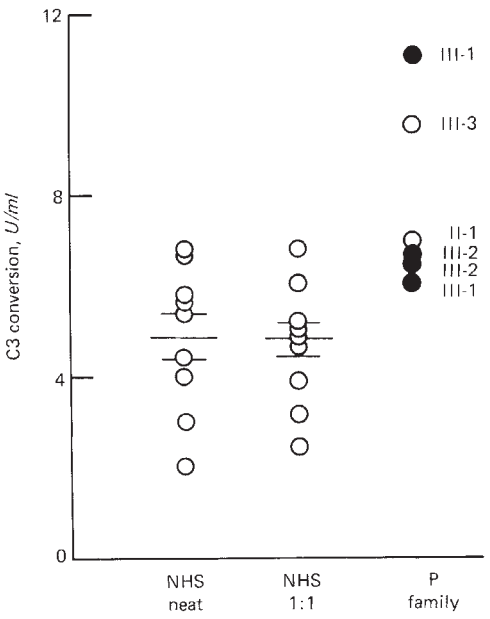


**Fig. 4.** *Effect of increasing the serum $Mg^{++}$ concentration on C3 conversion in specimens of serum from nine normal subjects, undiluted and diluted 1:1, and in serum from affected members of the P family.* Points for P family members are labeled with pedigree numbers; the *closed circles* indicate family members with glomerulonephritis. C3 conversion is quantitated by the loss of the B antigen of C3 in units per milliliter. The serum $Mg^{++}$ concentration was increased by 1.5 mM and incubation was for 20 min at 37°C. The *horizontal lines* indicate the means and SEM.

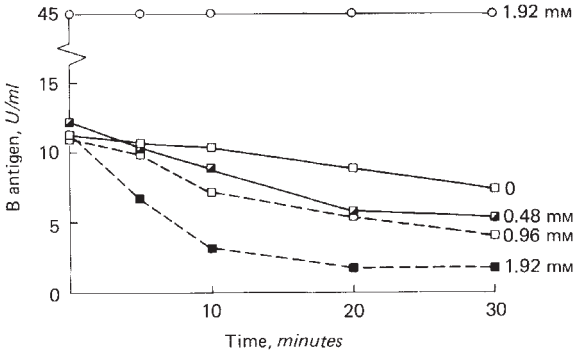


**Fig. 5.** *B antigen concentration after the $Mg^{++}$ concentration in normal serum* (circles) *and serum from the propositus* (squares) *increased by the amounts indicated and the serum incubated for various time intervals at 37°C.*

as a percent of initial level rather than as units per milliliter, C3 conversion with both activators was significantly greater in P family serum than in NHS. If the incubation was continued for longer than 5 min, B antigen loss in P family serum slowed and the values equaled those of the control.

Incubation of P family serum after the addition of $Mg^{++}$ resulted in more marked C3 conversion than was seen with other activators. Actual B antigen loss after the addition of sufficient 0.04 M $MgCl_2$ to raise the serum $Mg^{++}$ concentration by 1.5 mM and incubating at 37°C for 20 min is shown in Figure 4. Even expressed in units per milliliter, B antigen loss tended to be greater in P family than in either undiluted or diluted normal serum. Serum from family members without glomerulonephritis responded similarly to specimens from those with glomerulonephritis. Because the initial B antigen concentrations in all of these specimens were lower than those in diluted or undiluted NHS, B antigen loss expressed as a percent of initial level was much greater in P family serum.

The results of another experiment in which the serum of the propositus is incubated with various concentrations of $Mg^{++}$ is shown in Figure 5. In this experiment, incubation at 37°C after increasing the serum $Mg^{++}$ concentration by 1.92 mM had no effect on the C3 of normal serum but lowered the concentration of the B antigen of C3 by 85% in the serum of the propositus. Although the percentage fall was less when less $Mg^{++}$ was added, it is noteworthy that even without adding $Mg^{++}$ the B antigen level in the serum of the propositus fell by 35% in 30 min.

This response to complement activators suggested increased formation or persistence of a C3 convertase. Persistence of the amplification convertase, C3b,Bb, might be the result of a defect in the proteins regulating this convertase, H or I. Although both of these proteins were in normal concentration in P family serum (Table 1) and had normal mobilities by immunoelectrophoretic analysis, the possibility of a functional abnormality arose. To test this possibility, I, isolated from the serum of the propositus, was added back to a RI made from EDTA NHS. As shown in Figure 6, without the addition of I, marked cleavage of factor B occurred when $Mg^{++}$ was added. In this experiment, the amount of $Mg^{++}$ added was sufficient to raise the concentration in the reaction mixture by 0.68 mM. Addition of I before adding the $Mg^{++}$ inhibited factor B cleavage; normal I and I isolated from serum of the propositus were equally effective. Addition of $Mg^{++}$ also caused conversion of about 50% of C3 and inhibition of this conversion by added I was similar to the inhibition of factor B cleavage shown in Figure 6. Both C3 conversion and factor B cleavage were markedly inhibited at a concentration of either normal I or propositus I of about 0.3 mg/dl, about 8% of physiologic concentration. Thus, the I of the propositus was functionally normal.

The addition of $Mg^{++}$ to an RH made from EDTA-NHS resulted in C3 conversion of nearly 100% and moderate factor B cleavage. Because the RH contained residual EDTA, conversion was seen only after the RH $Mg^{++}$ concentration was increased by 2.4 mM by the addition of $MgCl_2$. In several RH preparations, H isolated from the serum of the propositus, from the serum of the propositus' brother (III-2), and from NHS were found to be equally effective in inhibiting C3 conversion and factor B cleavage (data not shown); the concentrations necessary for complete inhibition varied from 2 to 4 mg/dl, 4 to 8% of the normal level, in the various experiments.

Experiments which were the reverse of the above were also performed. Serum from the propositus, rather than NHS, was depleted of I, or H, and small quantities of the missing protein were added back. The results with propositus serum depleted of I and with NHS depleted of I were essentially the same; formation of C3b,Bb was similarly inhibited by increasing concentrations of normal I. Very different results were obtained with the RH. In two different RH preparations, one made from serum of the propositus and the other from the serum of his brother (III-2), a much higher concentration of H was required

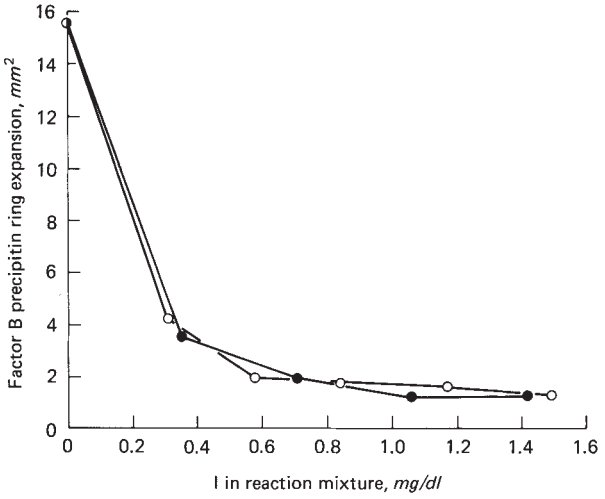


**Fig. 6.** *Effect of I in inhibiting factor B activation in an EDTA RI made from NHS after reconstitution with* $Mg^{++}$. The results with an I isolated from normal serum are indicated by the *open circles* and for an I isolated from the serum of the propositus, by the *closed circles*.

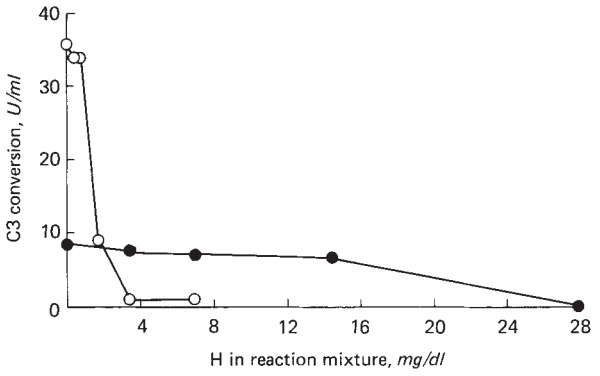


**Fig. 7.** *Effect of H isolated from normal serum in inhibiting C3 conversion, expressed on the ordinate as in Figure 4, after the addition of* $Mg^{++}$ *to two different EDTA RH preparations.* The results in the RH made from normal serum are indicated by the *open circles* and in an RH made from the serum of propositus, by the *solid circles*.

to prevent C3 conversion and factor B cleavage than was required by an RH made from NHS. The data for C3 conversion in the RH made from propositus serum are shown in Figures 7 and 8 and for factor B cleavage in Figure 9. The B antigen concentration of NHS depleted of H was 37 U/ml and, with incubation with $Mg^{++}$ alone, the concentration dropped to 1.2 U/ml, representing a C3 conversion of 35.8 U/ml or 97%. Thus, in Figure 7 C3 conversion at zero H concentration is plotted as 35.8 U/ml. With the addition of increasing amounts of H to the reaction mixture, C3 conversion diminished. Inhibition was virtually complete at an H concentration of 3.5 mg/ml. In the RH made from the serum of the propositus, the initial B antigen concentration was 8 U/ml, lower than for NHS, but again virtually all of this B antigen disappeared on the addition of $MG^{++}$. However, in this preparation, C3 conversion was not prevented by the addition of even four times the amount of H which prevented conversion in the RH made from normal serum.

To assess the contribution of P family C3 to the unusual requirement for H in this experiment, the C3 concentration of the propositus RH was increased by adding to it C3 isolated either from plasma of the propositus or normal human plasma. The C3 of the propositus raised the B antigen concentration of the propositus RH to 15.2 U/ml and the normal C3, to 21.8 U/ml. As shown in Figure 8, the addition of normal C3 enhanced the ability of H to inhibit C3 conversion as compared to the effect of adding C3 of the propositus. Thus, at an H concentration of 12 mg/dl, 27% of the C3 was converted in an RH fortified with normal C3 and 61% in an RH fortified with propositus C3.

As shown in Figure 9, data for factor B cleavage in these experiments verified that H was relatively ineffective in controlling the amplification convertase in an RH made from serum from the propositus. As with C3 conversion, inhibition of the factor B cleavage in the propositus RH required a much higher concentration of H than required for the normal serum RH. This requirement was not reduced by fortifying the RH with C3 from the propositus whereas the addition of normal C3 greatly increased the effectiveness of H in controlling cleavage. The results thus give no evidence of a defect in P family control proteins H and I but indicate, instead, that the amplification convertase, C3b,Bb, is resistant to control by H and that this is a consequence of an abnormality in P family C3.

The defect in P family C3 could not be correlated with an abnormal elecrophoretic mobility of this protein. Agarose gel electrophoresis of serum from II-1, II-2, III-1, III-2, and III-3 indicated that all were phenotypically C3S.

## Discussion

Combined heterozygous deficiency of C3 and factor B is unlikely as the cause of the complement abnormality in the P family since the structural genes for these two proteins are not linked. The structural gene governing factor B polymorphism is linked to HL-A [27] whereas that for C3 polymorphism is not [28, 29].

The abnormal response of the P family complement system to complement activators constitutes additional evidence against heterozygous deficiency. With both alternative and classical pathway activators there was a tendency for accelerated C3 conversion and factor B activation. The unequivocal acceleration of C3 breakdown produced by incubation with $Mg^{++}$ is a response which would not be expected with complement deficiency. It is well known that increasing the concentration of $Mg^{++}$ in serum causes, under certain circumstances, activation of the alternative pathway with C3 conversion and factor B cleavage [30, 31]. Kazatchkine, Fearon, and Austen [32] have shown that increasing $Mg^{++}$ concentration increases the affinity constant of factor B for C3b in a dose-dependent fashion without alteration of the affinity of H for C3b.

The greater C3 conversion in P family serum in response to an increased $Mg^{++}$ concentration could be a consequence of a greater content of the amplification C3 convertase, C3b,Bb. Formation of this convertase is initiated by $Mg^{++}$ dependent binding of factor B to C3b to form C3b,B. Cleavage of the bound factor B by D forms C3b,Bb and causes convertase activity to be expressed. Under normal conditions in vivo, or in serum in vitro, C3b is constantly being produced in small

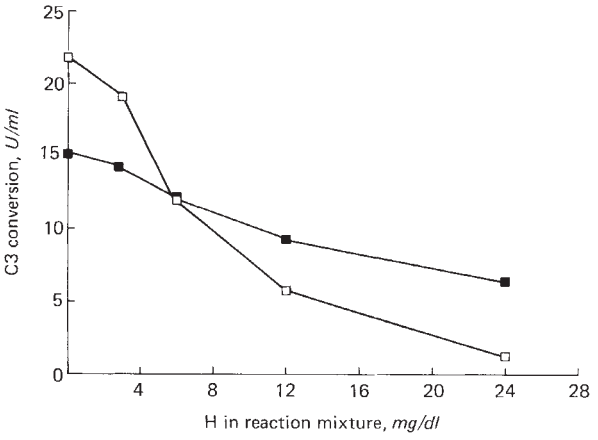


**Fig. 8.** *Effect of H isolated from normal serum in inhibiting C3 conversion after the addition of $Mg^{++}$ to an EDTA RH prepared from serum of the propositus to which either normal C3* (open squares) *or propositus C3* (closed squares) *was added.*

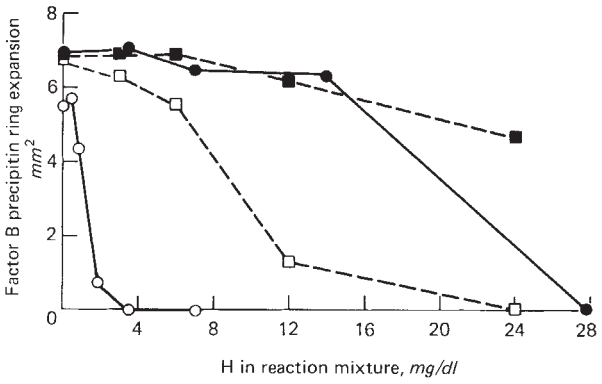


**Fig. 9.** *Effect of H isolated from normal serum in inhibiting factor B cleavage, expressed as factor B precipitin ring expansion, after the addition of $Mg^{++}$ to various EDTA RH preparations.* The results in an RH made from normal serum are indicated by the *open circles* and in an RH made from serum of the propositus, by the *closed circles*. Addition of normal C3 to the propositus RH increased the effectiveness of H in preventing factor B cleavage *(open squares)*, but the addition of propositus C3 had no effect *(closed squares)*.

quantities by a "priming convertase," C3Bb, thought to be assembled spontaneously by the interaction of C3, factor B and D [33, 34]. From the spontaneously made C3b, C3b,Bb can be formed which, by its convertase activity, creates more C3b. C3b,Bb is thus a constituent of a positive feedback loop normally controlled by the regulatory proteins. Part of this regulation is the rapid inactivation of C3b,Bb by displacement of Bb by H. Failure of inactivation of C3b,Bb would explain the accelerated C3 conversion produced by incubation of serum from the propositus at 37°C. The increase in C3 conversion produced by the addition of $Mg^{++}$ would be the result of increased C3b,B, and hence C3b,Bb, formation.

C3NeF in P family serum would cause persistence of the convertase activity of C3b,Bb, but it was not detectable. A functional abnormality in H or I, proteins which regulate the positive feedback, would reduce the rate of inactivation or increase the rate of formation of C3b,Bb, but these proteins were found to be functionally normal in that the concentrations required to prevent feedback turnover were the same for the P family proteins as for those isolated from NHS. Further experiments indicated that the increased convertase activity was the result of resistance of C3b,Bb to inactivation by H. This was demonstrated by the experiments with EDTA RH made from serum of the propositus or family member III-2. When an RH is reconstituted with $Mg^{++}$, C3b present in the serum forms with factor B the $Mg^{++}$ dependent complex, C3bB, which becomes an active convertase by cleavage of factor B by D, as noted above. Because H is absent in this preparation, this convertase activity of C3b,Bb is unopposed and through feedback cycling, the C3b,Bb concentration rapidly rises and C3 conversion is accelerated. As indicated in the present experiments, after reconstitution of an RH made from normal serum with $Mg^{++}$, concentrations of H which are 4 to 8% of the physiologic concentration will inactivate C3b,Bb and prevent feedback cycling. The fact that many times this amount of H is required to control feedback cycling in a P family RH after reconstitution with $Mg^{++}$ gives evidence that the C3b,Bb formed is resistant to inactivation by H. The fact that the H resistance persisted when C3 from the propositus was added to the RH but was partially corrected by adding normal C3 (Figs. 7 to 9) gives evidence that the defect is in C3. The resistance could be due to the fact that C3, as C3b, has a lower affinity for H or has a higher affinity for Bb, either of which would make H less effective. The pattern of inheritence implies that affected family members would be heterozygous for a gene producing this abnormal protein. The low C3 and factor B levels and the very negative C-R values would then be the result of spontaneous in vivo formation of C3b,Bb containing the abnormal C3b. This C3b,Bb would have an extended half-life because it would be less susceptible to intrinsic, H-mediated decay but, presumably, would still be susceptible to intrinsic decay.

The fact that both this complement abnormality and the nephritides found in the propositus and his brother are rare suggests a causal relationship. The basis for this relationship cannot be stated with certainty. C3 is involved in many events important in immune homeostasis including immune complex attachment to phagocytes [35, 36], immune complex solubilization [37], and modulation of the lymphocyte response to antigenic stimulation [38–40]. It is possible, for example, that the H resistance of P family C3b,Bb might affect the ability of the immune complexes bearing C3b to bind to phagocytes. Studies of the cellular C3b receptor [41] have shown that, when isolated, it reacts with C3b,Bb in a manner similar to H, displacing Bb and acting as a cofactor in the inactivation of C3b by I. Thus, if the P family defect was an abnormality of H binding to the abnormal C3b and if this H binding site is the same as that for the C3b receptor, affected family members might handle complexes abnormally. Because some of the C3b on the complexes would be unable to bind the C3b receptor, their clearance from the circulation might be slowed. Likewise, methods for the detection of complexes, such as those employing Raji cells, which depend on immobilization of complexes by their attachment to a cellular C3b receptor, might give spuriously low values and the optimum method would be one depending on Clq binding. However, the solid phase Clq method, as used in this study detected only slightly elevated levels of complexes in the propositus and his father.

Alternatively, it is possible that a persistently low C3 concentration in some way predisposes to glomerulonephritis. Pussell et al [12] reported a patient with heterozygous C3 deficiency and MPGN. A patient with chronic glomerulonephritis of immune complex origin reported by McLean et al [13] had an intermittently low C3 concentration and was found to be heterozygous for a hypomorphic variant of C3 (C3f). Wyatt et al [14] reported two families in which several members had low serum levels of C3 and factor B secondary to a partial deficiency of H. One of the family members had the hypocomplementemic vasculitis syndrome, two had IgA nephropathy, and another had hematuria and proteinuria of unknown origin. A survey of patients with MPGN seen in this institution has shown that an unusually high percentage has an inherited deficiency of one or more complement components. Thus, it is possible that persistently low levels of a component predispose to glomerulonephritis regardless of the nature of the defect responsible for the low level.

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